69. W. S. Broecker, testimony for U.S. Senate Subcommittee on Environmental Protection, 28 January 1987.
70. It is important to ask how long it might take the scientific community to be able to provide more credible time-evolving regional climatic anomaly forecasts from increasing greenhouse gases; S. H. Schneider, P. H. Gleick, L. Mearns in (*49*); Schneider *et al.* suggested that it will be at least 10 years and probably several decades before the current level of scientific effort can provide a widespread consensus on these details. The reason such time is needed at current levels of effort is that providing credible regional details will require the coupling of high resolution atmosphere, ocean, and sea ice models with ecological models that provide accurate fluxes of energy and water between atmosphere and land as well as nutrient cycling and chemical transformations that account for trace greenhouse gas buildup over time. A dedicated effort to accelerate the rate of progress could conceivably speed up the establishment of a consensus on regional issues, but at best 10 years or so will be necessary even with a dramatic effort. However, such efforts would clearly put future decision making on a firmer factual basis and help to make adaptation strategies more effective sooner.
71. The "tie-in" strategy was first formulated by E. Boulding, *et al.* [in *Carbon Dioxide Effects, Research and Assessment Program: Workshop on Environmental and Societal Consequences of a Possible CO_2-induced Climatic Change*, Report 009, CONF-7904143, U.S. Department of Energy (Government Printing Office, Washington, DC, October 1980), pp. 79–103]; it was later adopted by W. W. Kellogg and R. Schware, *Climate Change and Society, Consequences of Increasing Atmospheric Carbon Dioxide* (Westview Press, Boulder, CO, 1981).
72. S. H. Schneider and S. L. Thompson, in *The Global Possible: Resources, Development and the New Century*, R. Repetto, Ed., (Yale Univ. Press, New Haven, 1985), pp. 397–430.
73. J. A. Edmonds, W. B. Ashton, H. C. Cheng, and M. Steinberg (in preparation) have calculated that the U.S. contributes some 5% of CO_2 emissions, but this fraction could drop significantly if it holds emissions growth while other nations with large populations try to catch up with U.S. per capita energy use standards.
74. R. Revelle and H. Suess, *Tellus* **9**, 18 (1957).
75. K. E. Trenberth, G. W. Branstator, P. A. Arkin, *Science* **242**, 1640 (1988); S. H. Schneider, *Climatic Change* **13**, 113 (1988). Clearly, one hot year can no more prove that the greenhouse effect has been detected in the record any more than a few cold ones could disprove it. The 1990s, should they see a continuation of the sharp warming trend of the 1980s, will undoubtedly lead many more scientists to predict confidently that the increase in trace greenhouse gases has caused direct and clearly detectable climatic change. Already a few scientists are satisfied that the effects are 99% detectable in the record. J. Hansen (*1a*); see also, J. N. Wilford *New York Times*, 23 August 1988, p. C4.
76. *Toward an Understanding of Global Change, Initial Priorites for U.S. Contributions to the International Geosphere-Biosphere Program* (National Academy Press, Washington, DC, 1988); S. H. Schneider, *Issues Sci. Technol.* **IV** (no. 3), 93 (1988).
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Research Articles

Mechanism of Interleukin-2 Signaling: Mediation of Different Outcomes by a Single Receptor and Transduction Pathway

MICHAEL A. TIGGES, LESLIE S. CASEY, MARIAN ELLIOTT KOSHLAND

The T cell lymphokine, interleukin-2 (IL-2), plays a pivotal role in an immune response by stimulating antigen-activated B lymphocytes to progress through the cell cycle and to differentiate into antibody-secreting cells. An IL-2 inducible B lymphoma line, in which the growth and differentiation responses are uncoupled, provides a model system for dissecting the signaling mechanisms operating in each response. This system was used to show that both signals are initiated by IL-2 binding to a single, unifunctional receptor complex. Moreover, both signals are transduced by a pathway that does not involve any known second messenger system and that can be blocked by a second T cell lymphokine, interleukin 4. These findings suggest that the pleiotrophic effects of IL-2 are determined by different translations of the signal in the nucleus.

IN A PRIMARY IMMUNE RESPONSE A RESTING B LYMPHOCYTE IS triggered by antigen and T cell lymphokines to proliferate and to differentiate into a pentamer immunoglobulin M (IgM)–secreting cell. Although many of the lymphokines involved in the process have been identified, purified, and cloned, their precise roles in the response and the mechanism of their action have been difficult to define. The difficulties stem in part from the multifunctional nature of the lymphokines. Each can stimulate B cells, T cells, and in some cases accessory cells as well (*1*). Moreover, each lymphokine can deliver multiple signals to the same lymphocyte (*2–4*) and the effects can be enhanced or suppressed in the presence of other lymphokines (*5–8*). Difficulties also stem from the heterogeneity of normal lymphoid populations; even the most highly purified preparations are likely to be contaminated with other cell types, making the assignment of the lymphokine target difficult.

Understanding lymphokine signaling of B cells requires, therefore, a more defined experimental system in which the responses to a single pure lymphokine can be assessed in a cloned population of cells. We have recently developed such a system for analyzing the signals that the T cell lymphokine, interleukin-2 (IL-2), delivers to B cells in a primary immune response (*9*). The system makes use of a murine B cell line (BCL_1 CW13-3B3) that is representative of normal antigen-activated B cells. Like their normal counterparts (*6*), BCL_1 cells express IL-2 receptors and can be induced by IL-2 to assemble and secrete pentamer IgM (*9, 10*). The secretion of IgM is effected by the delivery of a single differentiative signal that activates the gene encoding the pentamer joining component, the J chain (*11*).

Unlike their normal counterparts, the BCL_1 lymphoma cells do not exhibit a proliferative response to IL-2 under the standard

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culture conditions used to elicit J chain gene expression. Their growth is rendered IL-2–dependent, however, if the amount of serum in the culture medium is reduced and the β-mercaptoethanol is omitted (*5*). Since the outcomes of the IL-2 signals can be dissociated in a clonal population of cells, the BCL_1 system provides a suitable model for examining the mechanisms by which each signal is relayed from the membrane to the nucleus. We now present evidence that the two signals are initiated by IL-2 binding to a common receptor and are transduced to the interior of the cell by a common pathway.

IL-2 signal input. As a first step in analyzing the signaling mechanism, the IL-2 receptors on BCL_1 3B3 cells were characterized by measuring the binding of recombinant ^{125}I-labeled IL-2 (rIL-2) and determining the number and affinity of receptors from Scatchard plots (Fig. 1). Assays of IL-2 binding to T and B lymphocytes have shown that activated cells express two forms of receptor molecules, a small number of high affinity and a much larger number of low affinity (*12, 13*). The BCL_1 subclone showed a similar pattern; the cells express an average of 800 receptors with an apparent dissociation constant (K_D) of 13 p*M* and 125,000 receptors with a K_D of 20 n*M*. Under the same binding conditions T lymphocytes from the IL-2–dependent CTLL line expressed 2100 high-affinity (K_D = 14 p*M*) and 500,000 low-affinity (K_D = 14 n*M*) receptors, in good agreement with data reported by others (*12, 14, 15*). Thus, the IL-2 receptors on BCL_1 cells and T cells are indistinguishable with respect to receptor-ligand interactions and differ only in numbers expressed.

The pattern of IL-2 binding to BCL_1 cells was correlated with signal outcome by titrating the differentiative and proliferative responses as a function of lymphokine dose. For the J chain induction experiments, BCL_1 cells were cultured in medium containing 10 percent fetal calf serum (FCS), 10^{-5}*M* β-mercaptoethanol, and increasing doses of rIL-2. The cells were stimulated for 72 hours, and RNA was extracted for dot blot hybridization assays with a J chain cDNA probe (Fig. 2A). The J chain RNA expression at each dose was quantified from densitometry of the dot hybridization films and plotted together with the high-affinity component of the IL-2 binding curve (Fig. 2B). Within the limits of precision of the densitometric measurements, it is clear that the dose response and high-affinity IL-2 binding curves are superimposable: strong evidence that the high-affinity receptor delivers the differentiative signal.

For the proliferation experiments, BCL_1 cells were cultured in medium containing 2 percent FCS, and increasing doses of IL-2 but no β-mercaptoethanol; after 20 hours, the extent of proliferation was determined by [^{3}H]thymidine incorporation assays. The growth curve obtained (Fig. 2C), like the dose response for J chain RNA induction, paralleled the interaction of IL-2 with the high-affinity receptors on BCL_1 cells. A similar correlation has been observed between IL-2 induced proliferation of T cells and the engagement of high-affinity receptors (*15*), suggesting that the IL-2 signals for growth and differentiation are initiated in B and T lymphocytes by a common mechanism.

Additional evidence for a common membrane signal was obtained by dissecting the high-affinity receptor on BCL_1 cells. The high-affinity receptor on T lymphocytes has been shown to be a bimolecular complex consisting of a 55-kD and a 75-kD chain. The 55-kD component is the well-defined Tac antigen which is expressed in large numbers on activated T and B cells and by itself binds IL-2 with a low affinity (K_D = 10 to 20 n*M*) (*12, 13, 16*). The 75-kD component has been identified by ^{125}I-labeled IL-2 crosslinking and found to be expressed in limited numbers on both resting and activated T cells (*17–19*). Alone the 75-kD chain binds IL-2 with an intermediate affinity (K_D = 0.5 to 1.0 n*M*) (*18*), but on the activated T cell membrane it interacts cooperatively with the 55-kD chain to generate the high-affinity receptor (*16, 18, 20*). Analyses of the function of each component show that the 75-kD chain transduces the T cell proliferation signal, whereas the 55-kD chain serves as a helper binding site (*16, 20*).

In view of these findings, the role of the 75-kD component in the BCL_1 high-affinity receptor was evaluated by the use of a monoclonal antibody that blocks the IL-2 interaction with the 55-kD chain (*21*). For such analyses, BCL_1 cells were stimulated with increasing doses of IL-2 in standard or low-serum medium containing the affinity-purified antibody in 500-fold molar excess of the p55 (the 55-kD protein) concentration. The J chain RNA content of the cells stimulated for 72 hours in standard medium was determined by the dot hybridization assays described above. The titrations (Fig. 3A) showed that J chain–specific transcripts were elicited in the presence of the antibody, but 50-fold higher IL-2 concentrations were required to match the response in control cultures lacking the antibody. Similar results were obtained when the proliferation of BCL_1 cells was assayed after 20 hours of stimulation in low-serum medium. The IL-2 concentrations required for half-maximal stimulation were 0.93 n*M* and 19 p*M* in the presence or absence, respectively, of the antibody to p55 (anti-p55). These shifts in the dose-response curves to concentrations characteristic of the intrinsic affinity of the 75-kD component provide strong presumptive evidence that the 75-kD chain alone can deliver the IL-2 signals to BCL_1 cells for J chain gene expression and proliferation.

The data from the dose-response titrations were substantiated by measuring the effects of anti-p55 on ^{125}I-labeled IL-2 binding to BCL_1 cells (Fig. 3B). In the presence of a 500-fold molar excess of antibody, only a low level of receptor interaction was detectable. The high-affinity binding component and most of the low-affinity

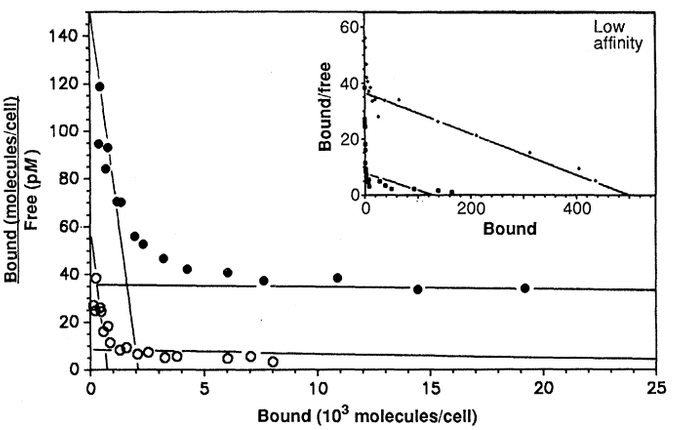

Fig. 1. Characterization of the IL-2 receptors on BCL_1 cells. For measurements of IL-2 binding, BCL_1 cells were washed twice with RPMI containing 10 percent fetal bovine serum (complete RPMI). Cells from the control IL-2–dependent T cell line, CTLL, were freed from bound IL-2 by three washings with warmed complete RPMI, 1 to 2 hours of incubation in the same medium at a concentration of 5×10^6 ml^{-1}, and two additional washings. Both preparations of cells were resuspended in complete RPMI at 2×10^7 ml^{-1} and 125 μl of each was added to an equal volume of ^{125}I-labeled rIL-2 (recombinant IL-2; specific activity, 1.5×10^6 cpm/pmole) diluted in serial twofold steps in complete RPMI containing 0.04 percent NaN_3 to final concentrations ranging from 200 n*M* to 3.6 p*M*. After the reaction mixtures were incubated for 1 hour at 22°C with continuous mixing, bound IL-2 was separated from free IL-2 by centrifugation through an oil layer (*15, 16, 19*), and the radioactivity in each fraction was determined to an accuracy of 1 percent in a gamma counter. Nonspecific binding was measured in quadruplicate samples containing 2.5 n*M* ^{125}I-labeled rIL-2 and a 500-fold excess of unlabeled IL-2. The number and affinity of receptors were determined from Scatchard plots (*38*) of the IL-2 binding data from two independent experiments after correction for nonspecific binding. Data from BCL_1 cells ○; from CTLL cells ●.

binding component were eliminated, indicating that the antibody effectively blocked both the formation of the high-affinity hybrid receptor and IL-2 interaction with the 55-kD receptor. The residual binding could, therefore, be attributed to IL-2 interaction with the free 75-kD receptor. Although the binding measurements were not sensitive enough to allow positive identification of these receptors, the finding that such a low level of receptor interaction was capable of inducing both differentiative and proliferative responses provides further evidence that the 75-kD chain is the transducer of the two signals.

IL-2 signal transduction. Coupling of the IL-2 high-affinity receptor to alternative signal transduction pathways is one mechanism by which a single membrane signal could generate different outcomes. This possibility was explored by examining the roles of known second messenger systems in the BCL_1 responses to IL-2. The phosphoinositide pathway was the first to be investigated because it is used by the antigen receptors on B and T cells (*22*) and has been implicated in the transduction of the IL-2 signal for T cell proliferation (*23*). In this pathway, ligand-induced perturbation of the receptor stimulates the hydrolysis of phosphatidylinositol 4,5-bisphosphate by phospholipase C. The hydrolysis yields two intracellular second messengers, inositol trisphosphate, which activates specific protein kinases by increasing cytosolic Ca^{2+}, and diacylglycerol, which binds to and directly activates protein kinase C (*22*).

The possibility that inositol trisphosphate mediates the IL-2 responses in BCL_1 cells was assessed from measurements of intracellular Ca^{2+}. BCL_1 cells containing the Ca^{2+}-sensitive dye, indo 1, were stimulated with IL-2, antibody to IgM (anti-IgM), and the Ca^{2+} ionophore ionomycin; changes in intracellular Ca^{2+} were determined by fluorometry (Fig. 4). Exposure of the cells to IL-2 at 200 U/ml had no significant effect on the Ca^{2+} concentration. The slight elevation observed was caused by fluorescent components in the medium used to dissolve the IL-2. The failure of IL-2 to elicit a Ca^{2+} response was not due to a block in the phosphoinositide pathway in BCL_1 cells. The subsequent addition of saturating amounts of anti-IgM induced an increase in intracellular Ca^{2+} concentration comparable to that reported for antigen receptor crosslinking in other B cell systems (*24–26*). The addition of ionomycin (100 ng/ml) had a more dramatic effect; the ionophore rapidly elevated the internal Ca^{2+} concentration 15-fold, saturating the dye, before cellular Ca^{2+} pumps began to reestablish an equilibrium.

The possibility of a second messenger role for diacylglycerol was assessed by treating BCL_1 cells with phorbol 12-myristate 13-acetate (PMA), which activates protein kinase C directly. BCL_1 cells were exposed either for 4 hours to PMA concentrations of 1 to 100 ng/ml, or for 24 hours to a constant amount of PMA (25 ng/ml) and increasing amounts of ionomycin (2 to 100 ng/ml). The ionomycin

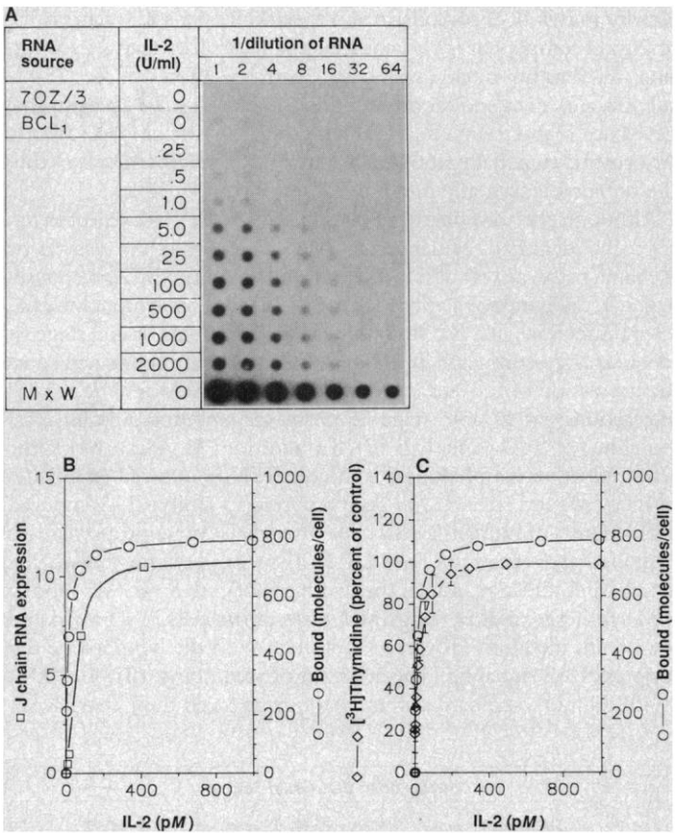

Fig. 2. Correlation of IL-2 binding to BCL_1 cells with J chain expression and proliferation. (**A**) Titration of J chain RNA response. Cytoplasmic RNA was extracted from BCL_1 cells (2×10^5 ml^{-1}) cultured for 3 days in the presence of increasing concentrations of rIL-2 (specific activity, 59 U/pmole, 4.0×10^6 U/ml). Serial twofold dilutions were dotted onto nitrocellulose and hybridized with a J chain cDNA probe (*9*). RNA from the 70Z/3 lymphoma and MxW 231.1a.2 hybridoma lines provided negative and positive controls, respectively. The first dot represents 5×10^6 cell equivalents. (**B**) Dose response curve for J chain RNA. The level of J chain expression was determined from densitometer traces of the dot hybridization films, normalized to 1 U of density per 1 U of IL-2 per milliliter (□----□) and plotted with the high affinity binding component of the IL-2 binding curve (○----○). (**C**) Dose response curve for BCL_1 proliferation. BCL_1 cells were washed and resuspended at a density of 4×10^5 ml^{-1} in low-serum medium (RPMI containing 2 percent fetal bovine serum and no β-mercaptoethanol) (*5*). Triplicate samples (0.1 ml) were cultured for 20 hours with equal volumes of increasing concentration of IL-2 and then treated for 4 hours with 1 μCi of [^{3}H]thymidine (Amersham, 5 Ci/mmole). The [^{3}H]thymidine incorporated was determined by standard assay, corrected for the number of counts per minute (cpm) incorporated in the absence of IL-2, and expressed as the percentage of the average maximum incorporation. The average values of three separate experiments (◇----◇) with standard error bars were plotted with the high affinity binding component of the IL-2 binding curve (○----○).

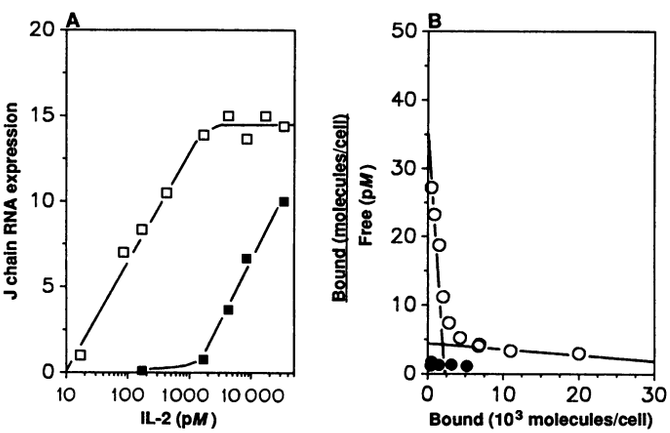

Fig. 3. Effect of anti-p55 on IL-2 induction of J chain RNA expression. (**A**) Titration of J chain RNA response, BCL_1 cells (2×10^5 ml^{-1}) were cultured with increasing concentrations of rIL-2 in the presence or absence of saturating amounts of the monoclonal antibody, PC61, specific for the 55-kD chain of the murine IL-2 receptor (*21*). The antibody was purified from hybridoma supernatants by affinity chromatography on protein A–Sepharose (Bio-Rad, Affigel) and used at a concentration of 8 μg/ml. The cells were cultured for 72 hours and RNA was isolated as described in the legend to Fig. 2. The data for the dose response curves for J chain RNA expression in the absence (□----□) or presence (■----■) of monoclonal antibody to p55 (anti-p55 mAb) were obtained as described in the legend to Fig. 2. (**B**) Scatchard plots of IL-2 binding to BCL_1 cells assayed in the absence (○----○) or presence (●----●) of anti-p55 mAb at 40 μg/ml by the procedures described in the legend to Fig. 1.

was included to ensure that enough free Ca^{2+} was available for protein kinase C activation. The effects of these treatments were assayed on cells cultured in standard or low-serum medium with and without IL-2 (200 U/ml).

Dot hybridization analyses of J chain RNA content (Fig. 5) showed that neither PMA alone nor the combination of PMA and ionomycin could mimic the IL-2 signal. The failure of these agents to induce transcription of the J chain gene could not be attributed to toxic effects since the doses used did not significantly alter the capacity of BCL_1 cells to respond to IL-2 (Fig. 5). Moreover, the same treatments were effective in stimulating EL-4 T lymphoma cells to produce IL-2, a response that is mediated via the phosphoinositide pathway (*27*). Assays of [^{3}H]thymidine uptake gave similar results (Table 1). Neither PMA nor ionomycin elicited a proliferative response. At doses below 10 ng/ml, these reagents had no effect on the growth rate of BCL_1 cells; at higher doses both reagents became increasingly inhibitory. Although these experiments do not exclude the possibility that IL-2 binding to BCL_1 cells activates protein kinase C, they do rule out diacylglycerol as a second messenger for IL-2–induced transcription of the J chain gene and IL-2–induced proliferation.

The combined data from the Ca^{2+} and PMA experiments indicate that the high-affinity IL-2 receptors expressed by B cells are not coupled to the phosphoinositide pathway. Thus, IL-2 signals that are generated late in a primary immune response are transduced by a different route from that of the initial antigen signal. The shift to a different transduction system could serve as a mechanism for maintaining separate control of the early and late events in the response. Whether a similar shift occurs during the development of immune T cells remains to be determined. IL-2 binding to T cells has been reported to stimulate phosphoinositol turnover and translocation of protein kinase C to the membrane, but a direct relation between these effects and IL-2–induced proliferation of T cells has not been established (*23*).

Subsequent analyses of two other potential second messengers, cyclic AMP and cyclic GMP, also gave negative results. The BCL_1 responses to IL-2 are not affected by inhibitors, such as *N*-[2-(methylamino)-ethyl]-5-isoquinoline-sulfonamide dihydrochloride (H8), which interact directly with cyclic AMP– and cyclic GMP–dependent protein kinases (*28*). Moreover, both responses are insensitive to cholera and pertussis toxins (*29*), which modify specific GTP-binding proteins involved in the regulation of cyclic AMP and cyclic GMP (*30*). Thus, none of the well-defined second messenger–dependent protein kinases appears to play a role in IL-2 signaling of BCL_1 growth and differentiation. The elimination of these classical systems leaves two alternative possibilities: the IL-2 signals could be transduced either by an unknown second messenger–dependent pathway or by direct activation of a protein kinase activity associated with the receptor itself. The latter possibility is supported by a report that the IL-2 receptor complex contains a protein kinase activity that is IL-2 responsive and specifically phosphorylates a 75- to 78-kD component (*31*). Positive identification of such a transduction mechanism must await the characterization of the 75-kD subunit as well as several other higher molecular mass proteins that have been found to be associated with the receptor complex (*17, 32*). At present, essentially nothing is known of the structure, and thus the potential enzymatic function, of these components.

Although the transduction system used by the IL-2 receptor has yet to be identified, evidence that both IL-2 signals are relayed by the same pathway was obtained from analyses of the BCL_1 responses to the T cell lymphokine interleukin-4 (IL-4). This lymphokine has several functions in a B cell immune response. At the initial stages it serves as a co-stimulant in antigen-driven activation and enhances the expression of cell surface Ia molecules required for T helper cell interactions; at a later stage it promotes immunoglobulin class switching to IgG_1 and IgE (*3*). In addition, IL-4 can block the action of other lymphokines; it inhibits IFN-γ–induced expression of IgG_{2a} (*3*) and recently has been reported to inhibit IL-2–induced proliferation of human B cells (*33*). In view of these properties, we examined the effects of IL-4 on BCL_1 cells. Treatment with the lymphokine did not induce the synthesis of J chain RNA, modify IgM synthesis, or alter the growth rates of the cells in standard and low-serum medium. However, treatment with the lymphokine did suppress IL-2 signaling. The addition of recombinant IL-4 (rIL-4) at 400 U/ml to standard culture medium reduced the J chain RNA

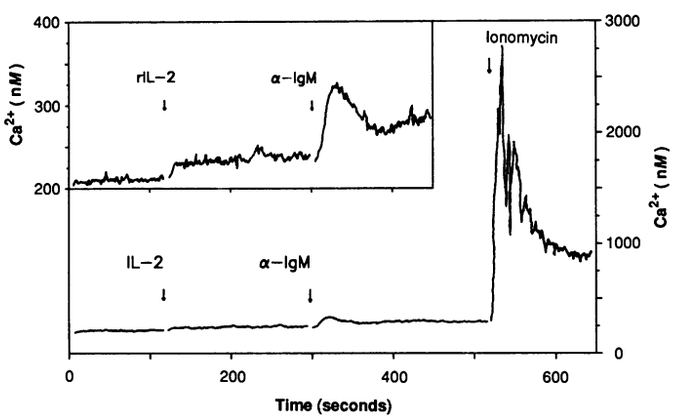

Fig. 4. Failure of IL-2 to induce Ca^{2+} mobilization in BCL_1 cells. BCL_1 cells (2×10^7 ml^{-1}) were treated with an excess of the acetoxymethyl ester of Indo-1 (Molecular Probes, Eugene, Oregon) by incubation for 20 minutes at 37°C in the presence of 2.5 μ*M* dye and incubation for another 20 minutes after the sample was diluted 1/10 with warmed complete RPMI. The cells were then washed with warmed RPMI containing 5 percent serum but no phenol red or pyroxidine HCl, warmed to 37°C in the dark, and placed in a temperature-controlled and stirred quartz cuvette. The effects of the successive addition of IL-2 (200 U/ml), goat antiserum to mouse IgM (5 μg/ml) and ionomycin (100 ng/ml) on Indo-1 fluorescence (excitation 334 nm, emission 400 nm) were measured with a Spex F2C spectrofluorometer (Spex Industries, Edison, New Jersey). The fluorescence maximum was determined after the cells were lysed with digitonin (5 μg/ml) and the fluorescence minimum after the addition of excess EGTA. The concentration of free Ca^{2+} was calculated from the fluorescence values as described (*26, 39*).

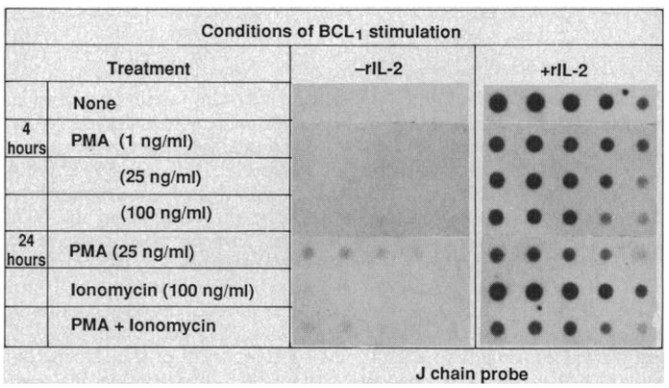

Fig. 5. Failure of a protein kinase C–activating phorbol ester or a Ca^{2+} inophore (or both) to induce J chain RNA expression in BCL_1 cells. BCL_1 cells (2×10^5 ml^{-1}) were treated for 4 hours with PMA at the concentrations indicated in the absence or presence of IL-2 at 200 U/ml. In a separate experiment, BCL_1 cells were exposed for 24 hours to the Ca^{2+} ionophore, ionomycin, alone, PMA alone, or both agents at the concentrations indicated. At the end of the treatment period in both experiments, the cells were washed twice with warmed balanced salt solution containing 5 percent fetal bovine serum and replated in complete RPMI with and without IL-2 (200 U/ml). After the cells were cultured for 72 hours, cytoplasmic RNA was isolated for dot hybridization as described in the legend to Fig. 2.

response to IL-2 (200 U/ml) at least 30-fold (Fig. 6A). Similarly, addition of rIL-4 at 400 U/ml to low-serum medium inhibited the proliferative response completely at low IL-2 doses and partially at higher doses (Fig. 6B). The suppression is not effected by down-regulation of the IL-2 receptor or by competitive inhibition of the IL-2 receptor by IL-4. Measurements of IL-2 binding to BCL_1 cells before and after the addition of IL-4 gave indistinguishable patterns (Fig. 6C). Moreover, once the IL-4 was withdrawn from the culture medium, the BCL_1 cells displayed the typical IL-2 responses. These findings indicate that IL-4 acts at a subsequent common step in the IL-2 signaling process. The most likely mechanism is that an IL-4–generated signal interferes with the system used by the IL-2 receptor to transduce both the differentiative and proliferative signals. This interpretation is supported by studies (*34*) showing that IL-4 binding to its receptor, like IL-2 binding to its receptor, does not induce inositol lipid metabolism, Ca^{2+} mobilization, or protein kinase C translocation.

Implications for the mechanism of IL-2 signaling. The multiple effects that a single lymphoid hormone exerts on a single target population and the modulation of those effects by other lymphokines poses a serious problem for understanding the mechanisms of hormone signaling. How does the target cell integrate the diverse information received from specific receptors and translate that information into different outcomes? Various solutions to the problems have been proposed, among them multifunctional receptors, "crosstalk" between different receptors, the use of alternative transduction pathways, and differential translation of the signal in the nucleus (*35*). We have explored these possibilities by examining the mechanisms of IL-2 signaling in a B lymphoma line where the two signal outcomes, growth and differentiation to J chain gene expression, can be uncoupled by the use of different culture conditions.

Analysis of signal input to the lymphoma cells showed that high-affinity IL-2 receptors mediate both responses. These receptors are a complex of two IL-2 binding proteins, a 75-kD and a 55-kD chain that interact cooperatively to generate a single high-affinity site that is capable of binding IL-2 at the picomolar concentrations found physiologically. Dissection of receptor function revealed that the 75-kD chain is responsible for transducing the signals for proliferation and J chain gene activation, whereas the 55-kD chain serves to amplify the affinity of binding. IL-2 signal input to T cells has been shown to operate by a similar mechanism (*16*), indicating that a single unifunctional receptor initiates all IL-2–induced responses in lymphocytes.

Analyses of signal transduction in the lymphoma cells indicated that a single pathway is also used to transmit the IL-2 messages from the high-affinity receptor to the interior of the cell. The evidence was derived, in part, from the examination of conventional second messenger systems. Of those tested, Ca^{2+} flux, inositol trisphosphate, and diacylglycerol production, cyclic AMP– and cyclic GMP–dependent protein kinases, none was found to act as an intermediate in IL-2–induced proliferation or in IL-2–induced activation of the J chain gene. These findings limited the possible transduction system for both responses to a receptor-associated protein kinase or an unknown second messenger system.

More direct evidence for a common transduction pathway was obtained from the inhibition of both IL-2 responses by the lymphoid hormone, IL-4. No "crosstalk" between the lymphoma IL-4 and IL-2 receptors was observed; in the presence of IL-4 neither the number nor the binding properties of the high-affinity IL-2 receptors were altered. The antagonistic action of IL-4 is, therefore, exerted at a subsequent common step in the IL-2 signaling process, the most likely candidate being the transduction pathway. The suppressive effect of IL-4 observed with BCL_1 cells may reflect regulatory events operating in a normal B cell immune response. It is possible that competition between IL-4 and IL-2 signals determines whether the activated B lymphocytes become committed to pentamer IgM synthesis or switches to the synthesis of monomer IgG_1 and IgE. Moreover, suppression of IL-2 responses by IL-4 would explain earlier observations that in the presence of T cell superna-

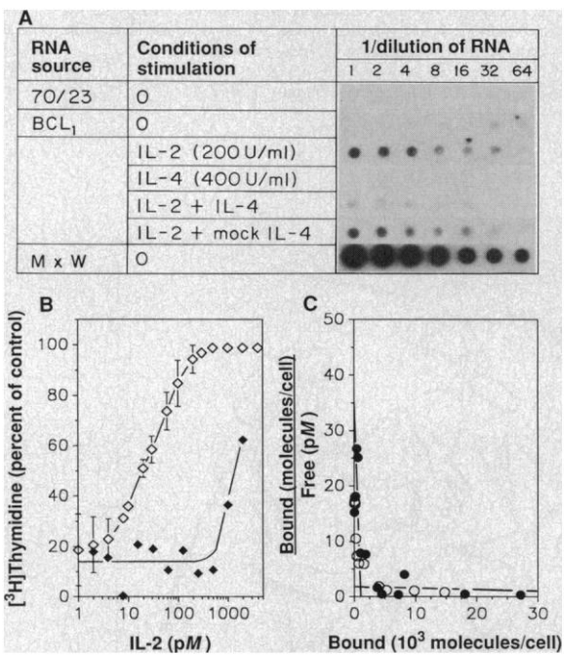

Fig. 6. Inhibition by IL-4 of the BCL_1 responses to IL-2. (**A**) Titration of J chain RNA response. BCL_1 cells (2×10^5 ml^{-1}) were cultured with IL-2 or a COS supernatant containing rIL-4. The mock IL-4 treatment consisted of an equal volume of COS supernatant lacking rIL-4. After 72 hours of cell culture, cytoplasmic RNA was isolated for dot hybridization as described in the legend to Fig. 2 (**B**) Dose response curves of BCL_1 proliferation in the absence (◇----◇) and presence (◆----◆) of rIL-4 at 400 U/ml. The proliferation assays were performed as described in the legend to Fig. 2. (**C**) Scatchard plots of IL-2 binding to BCL_1 cells assayed in the absence (○) and presence (●) of rIL-4 (400 U/ml) by the procedures given in the legend to Fig. 1.

Table 1. BCL_1 proliferation in low serum media. Proliferation assays were performed as described in the legend to Fig. 1 except that the RPMI contained 3 percent instead of 2 percent fetal bovine serum. The higher serum concentration increased the [^{3}H]thymidine incorporation in the untreated cells by a factor of 5.

Treatment	Dose (mg/ml)	[^{3}H]Thymidine incorporated* (cpm)
10 percent fetal calf serum		
None		77,834†
IL-2‡	50.8	75,269†
3 percent fetal calf serum		
None		27,834†
IL-2	50.8	45,014 (4534)
PMA	1	29,399 (126)
	25	16,735 (973)
	100	14,891 (1947)
Ionomycin	10	27,267 (2284)
	100	8,806 (1855)
PMA }	25	
Ionomycin }	100	7,913 (1072)

*The incorporation values are the averages of triplicate determinations and the numbers in parentheses are the standard errors. †The incorporation value is from a single determination. ‡An IL-2 concentration of 50.8 ng/ml is 200 U/ml.

tants, large doses of IL-2 are required to drive activated B lymphocytes to J chain expression and high-rate pentamer IgM secretion (*6*).

On the basis of these analyses, it appears that the IL-2 signals for lymphoma growth and differentiation are read and translated into different outcomes in the nucleus. A few clues to the mechanisms involved have been obtained in the case of J chain gene activation. Transcription of the gene has been shown to correlate with the development of a 5′ hypersensitive site and a change in protein binding to that region (*9, 36*). In contrast, essentially nothing is known of the events that regulate proliferation. The control processes are clearly more complex as the serum dependency of the BCL_1 proliferative response illustrates. With the advantages of the lymphoma model system, however, it should be possible to decipher the mechanisms of signal integration. Moreover, since IL-2 plays a similar vital role in T cell immune responses, the information gained from the lymphoma model should contribute to the understanding of IL-2 signaling action in these cells as well.

Note added in proof: After this article was submitted, Mills *et al.* and Valge *et al.* reported that protein kinase C activation is not essential for IL-2–induced proliferation of T cells (*37*).

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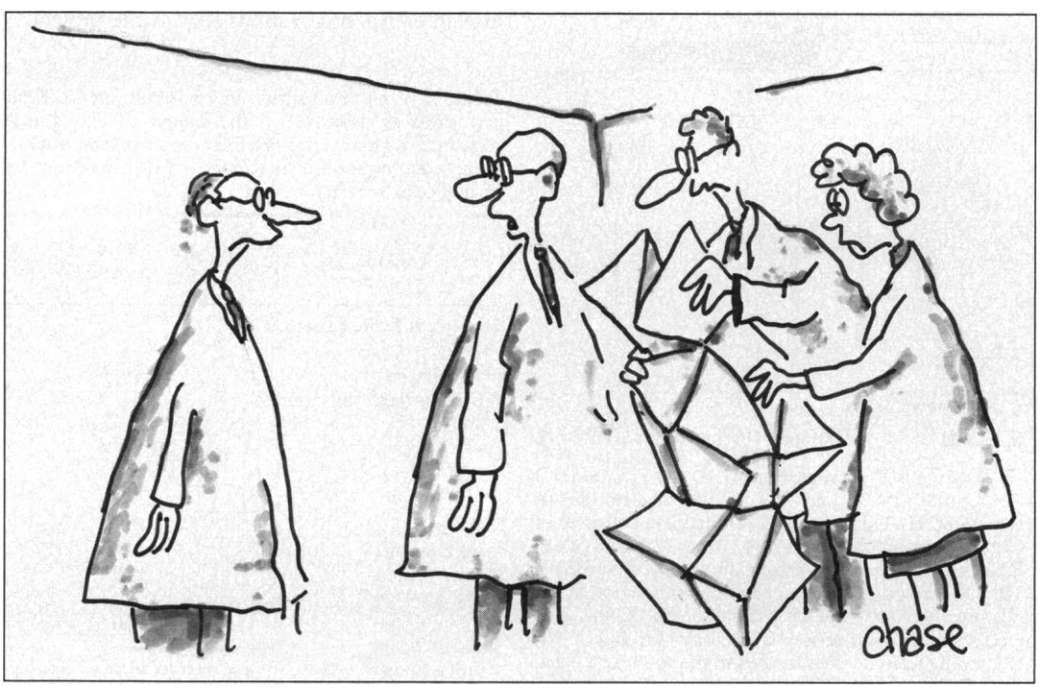

"We finished the genome map, now we can't figure out how to fold it!"